124. On the Hyperpolarizing Inactivation of the Ca\(^{++}\)-dependent Action Potentials

By Shizuko Iwasaki, Youko Satow, and Toshiko Kuroda
Department of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo
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It has been known that the degree of sodium activation depends on the membrane potential level conditioned (Hodgkin and Huxley, 1952). Namely, it is inactivated when the membrane potential is depolarized and activated to some extent when the membrane potential is hyperpolarized. This characteristic membrane potential dependency of the Na activation appears to be general in many excitable membrane where the Na ions play a principal role for developing the action potentials.

In the soma membrane of the X-organ of crayfish, spike potentials resistant to Tetrodotoxin (TTX) was observed (Iwasaki and Satow, 1971). Their amplitude changed as the Ca ion concentration of the medium was altered. In the present short report, inactivation phenomenon of the Ca\(^{++}\)-dependent spike potentials by the membrane potential conditioned was investigated.

The X-organ of the crayfish is a cluster of neurosecretory cell soma which is located on the medulla terminalis of the eye stalk. The some characteristic nature of the soma membrane has been reported elsewhere (Iwasaki and Satow, 1971, 1973). In the most respects, the apparatus and the method were similar to those described by Iwasaki and Satow (1971). Tetrodotoxin (Sankyo Co.) 10\(^{-7}\) g/ml and Tetraethylammonium chloride (TEA) (Eastman Kodak Co.) were used in the experiment.

In the crayfish solution, the membrane potential was shifted with the injected current passed through the electrode by means of the bridge circuit. Action potentials (upper) elicited by the superposed depolarizing pulses of 50 msec in duration and their first derivatives (lower) were arranged with varied membrane potential levels in Fig. 1, AI. The broken line was drown at the level of resting membrane potential. The active membrane potential and the maximum rate of rise of the spike potentials were plotted against the membrane potential level conditioned in Fig. 1, B. For convenience the minus sign in the membrane potentials was omitted. TTX lowered the max. rate of rise by a factor of 4–5 if it is compared at each maximum and decreased the spike height.
Fig. 1. Initiation of action potentials in the TTX-medium and its membrane potential dependency. A1: Action potentials (upper) and their first derivatives (lower) were arranged with the conditioned membrane potentials. The membrane was conditioned at the new level over than 10 sec. Broken line was drawn at the resting potential level. AII: In the TTX medium (10^{-7} g/ml), action potentials and their first derivatives were arranged as above figures. B: The maximum rate of rise of the action potentials and the active membrane potentials were plotted against the membrane potential level conditioned. The experiments were performed in the following order; normal crayfish solution (open circle), TTX containing solution (filled circle) and normal solution (triangle). At the hyperpolarized membrane potentials, duration of action potentials decreased characteristically in the normal medium. Different symbols were adopted to those narrow action potentials. In the TTX medium, no action potentials could be elicited at the membrane potentials over than 60 mV in this preparation. Resting potential was 47 mV at the beginning and 43 mV at the end of the experiment.
When the membrane was depolarized and kept the potential level for over ten seconds, the peak level of the action potential elicited by a short depolarizing pulse lowered and its rate of rise decreased as the case of that in other excitable membrane where Na ions work principally. When the membrane was hyperpolarized, the development of the action potential seemed to be retarded. Decrease in the spike height and the max. rate of rise were generally observed. When the membrane was hyperpolarized strongly, action potential could not be elicited by any depolarizing current pulses. The potential change indicating “delayed rectification” was observed during the stimulating current. This suggests the development of the potassium permeability increase during the depolarizing pulse. It appears that there is optimum membrane potential for the development of the Ca\(^{++}\)-dependent action potential in the TTX medium. The optimum membrane potential was varied from one cell to another. It is not always true that the max. rate of rise is maximum at the resting potential level.

Sr\(^{++}\) can substitute for Ca\(^{++}\) in the membrane of the X-organ. Action potential can be elicited in the Sr\(^{++}\)-TTX medium and also showed the same behavior against the membrane potential level conditioned as in the Ca\(^{++}\)-TTX solution.

Threshold membrane potential for the delayed rectification in the medium which contains TTX and Mn\(^{++}\) (10 mM) was larger (more negative) when the membrane potential was equilibrated at the hyperpolarized level over than 10 sec. From the current-voltage relationship in this medium, it can be suggested that at the hyperpolarized membrane potential level the potassium activation is more remarkable than that at the resting membrane potential level (unpublished data), as the case of squid giant axon (Hodgkin and Huxley, 1952). If one assume that the amount of activation could be estimated from the max. rate of rise of the action potential, Ca\(^{++}\) activation might be 1/4 – 1/5 of the Na activation. Furthermore, threshold membrane potential of the Ca spike is less negative than that of Na spike by 10–20 mV in the X-organ cell soma and close to the threshold of delayed rectification (Fig. 3, in Iwasaki and Satow, 1971). It is expectable, from the lines of circumstantial evidence, that the potassium activation may interfere with the activation of Ca dependent spike potentials.

The potassium activation is suppressed by an application of Tetraethylammonium chloride (TEA) to the excitable membrane externally or internally (Hagiwara and Watanabe, 1955; Hille, 1967; Armstrong and Hille, 1972). The application of TEA 10–200 mM to the X-organ cell soma brought about the increase in the spike
height, increase in the duration of action potentials and decrease in the threshold depolarization. This indicates a suppression of K activation by this drug. The hyperpolarizing inactivation, at the same time, became less pronounced. In some preparations, it disappeared (Fig. 2). The max. rate of rise of the action potential was unchanged throughout the hyperpolarized membrane potential level examined (Fig. 2, B).

When the membrane potential was shifted to the hyperpolarized level in the TTX medium, it took several hundred milliseconds to

Fig. 2. Effect of TEA on the hyperpolarizing inactivation of Ca-spike. AI: Action potentials (upper) and their first derivatives (lower) were arranged with the membrane potential level conditioned. AII: TEA (50 mM) was added to the TTX medium. Note the development of action potentials at the hyperpolarized membrane potential level. In the extreme left figure, the conditioned potential level was out of the record. Calibration: 50 mV and 25 msec for AI, 50 mV and 50 msec for AII. B: The active membrane potentials and the max. rate of rise were plotted against the membrane potential level conditioned. The disappearance of the hyperpolarizing inactivation of Ca**+-dependent spike potentials is evident.
establish the hyperpolarizing inactivation. The change of the membrane potential in the opposite direction from this level brought about the disappearance of the hyperpolarizing inactivation. It took several hundred milliseconds to several seconds. The latter, in general, took more time than the former, although the half time to establish the new activation level was varied depending on the potential level to be attained. The slope resistance which is measured by the ratio of the potential change to the current applied changed after sudden shift of the membrane potential. It has the similar time course to that of the activation estimated from the max. rate of rise of the action potentials.

From the present experiment, it can be concluded that the hyperpolarizing inactivation of the Ca**+-dependent spike potentials in the soma membrane of the X-organ, for the most part, must be due to the simultaneous increase in the potassium permeability which impedes the activation of Ca**+-dependent spike potentials. Decrease in the Ca current at the hyperpolarized membrane potential level in the ganglion cells of Aplysia was reported (Geduldig and Gruener, 1970). The suppression of the action potential at the hyperpolarized membrane potential, seemingly, is characteristic in the Ca**+-dependent spike potential and may be attributable to the interference by the potassium activation.

References


